

CLAIMS

1. A chromoprotein derived from *Anthopleura inornata* having the following properties:

- (1) the absorption maximum wavelength is 605 nm;
- (2) the molar absorption coefficient is 47,550 at 605 nm; and
- (3) the pH sensitivity of light-absorbing property is stable at between pH 5 and pH 10.

2. A chromoprotein having either one of the following amino acid sequences:

- (a) the amino acid sequence shown in SEQ ID NO: 1; and
- (b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and having light-absorbing properties.

3. A chromoprotein derived from *Anthopleura inornata* having the following properties:

- (1) the absorption maximum wavelength is 553 nm;
- (2) the molar absorption coefficient is 25,300 at 553 nm; and
- (3) the pH sensitivity of light-absorbing property is stable at between pH 5 and pH 10.

4. A chromoprotein having either one of the following amino acid sequences:

- (a) the amino acid sequence shown in SEQ ID NO: 3; and
- (b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 3, and having light-absorbing properties.

5. A DNA encoding the protein of any of claims 1 to 4.

6. A DNA of either one of followings:

- (a) DNA encoding the amino acid sequence shown in SEQ ID NO: 1; and
- (b) DNA encoding a protein which has an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and has light-absorbing properties.

7. A DNA of either one of followings:

- (a) DNA encoding the amino acid sequence shown in SEQ ID NO: 3; and
- (b) DNA encoding a protein which has an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 3, and has light-absorbing properties.

8. A DNA having either one of the following nucleotide sequences:

- (a) the nucleotide sequence shown in SEQ ID NO: 2; and
- (b) a nucleotide sequence comprising a deletion, substitution and/or addition of one or several nucleotides with respect to the nucleotide sequence shown in SEQ ID NO: 2, and encoding a protein having light-absorbing properties.

9. A DNA having either one of the following nucleotide sequences:

- (a) the nucleotide sequence shown in SEQ ID NO: 4; and
- (b) a nucleotide sequence comprising a deletion, substitution and/or addition of one or several nucleotides with respect to the nucleotide sequence shown in SEQ ID NO: 4, and encoding a protein having light-absorbing properties.

10. A recombinant vector having the DNA of any of claims 6 to 9.

11. A transformant having the DNA of any of claims 6 to 10 or the recombinant vector of claim 10.

12. A fusion protein composed of the chromoprotein of any of claims 1 to 4 and another protein.

13. A method for analyzing a physiologically active substance, which is characterized in that the FRET (fluorescence resonance energy transfer) method is applied using the chromoprotein of any of claims 1 to 4 as an acceptor protein.

14. A light-absorbing reagent kit comprising the chromoprotein of any of claims 1 to 4, the DNA of any of claims 5 to 9, the recombinant vector of claim 10, the transformant of claim 11, or the fusion protein of claim 12.

15. A fluorescent protein derived from *Trachyphyllia geoffroyi*, which has the following properties:

(1) the color is changed from green to red by irradiation of ultraviolet ray; the excitation maximum wavelength is 508 nm (green) and 572 nm (red); and the fluorescence

maximum wavelength is 518 nm (green) and 581 nm (red);

(2) the molar absorption coefficient (green) at 508 nm is $98800 \text{ M}^{-1}\text{cm}^{-1}$; and the molar absorption coefficient (red) at 572 nm is $60400 \text{ M}^{-1}\text{cm}^{-1}$;

(3) the quantum yield is 0.80 (green) and 0.33 (red); and

(4) pKa regarding the pH sensitivity of the green and red are both 5.7.

16. A fluorescent protein having either one of the following amino acid sequences:

(a) an amino acid sequence shown in SEQ ID NO: 5; or

(b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 5, and having fluorescent properties.

17. A fluorescent protein having an amino acid sequence shown in SEQ ID NO: 7.

18. A fluorescent protein derived from *Scolymia vitiensis*, which has the following properties:

(1) the color is changed from green to red by irradiation of ultraviolet ray; the excitation maximum wavelength is 508 nm (green) and 578 nm (red); and the fluorescence maximum wavelength is 518 nm (green) and 588 nm (red);

(2) the molar absorption coefficient (green) at 508 nm is $102250 \text{ M}^{-1}\text{cm}^{-1}$; and the molar absorption coefficient (red) at 578 nm is $76950 \text{ M}^{-1}\text{cm}^{-1}$;

(3) the quantum yield (fluorescence) is 0.43 (green) and 0.51 (red); and

(4) pKa regarding the pH sensitivity of the green (508 nm) is 5.8; and pKa regarding the pH sensitivity of the red (578 nm) is 6.5.

19. A fluorescent protein having either one of the following amino acid sequences:

(a) an amino acid sequence shown in SEQ ID NO: 9; or

(b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 9, and having fluorescent properties.

20. A fluorescent protein having an amino acid sequence shown in any of SEQ ID NO: 11, 13, 15 or 17.

21. A DNA of either one of the following:

- (a) DNA which encodes the amino acid sequence shown in SEQ ID NO: 5; or
- (b) DNA which encodes an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 5, and encodes a fluorescent protein:
- (c) DNA having a nucleotide sequence shown in SEQ ID NO: 6; or
- (d) DNA having a nucleotide sequence comprising a deletion, substitution and/or addition of one or several nucleotides with respect to the nucleotide sequence shown in SEQ ID NO: 6, and encoding a fluorescent protein.

22. A DNA of either one of the following:

- (a) DNA which encodes the amino acid sequence shown in SEQ ID NO: 7; or
- (b) DNA having a nucleotide sequence shown in SEQ ID NO: 8.

23. A DNA of either one of the following:

- (a) DNA which encodes the amino acid sequence shown in SEQ ID NO: 9; or
- (b) DNA which encodes an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 9, and encodes a fluorescent protein:
- (c) DNA having a nucleotide sequence shown in SEQ ID NO: 10; or
- (d) DNA having a nucleotide sequence comprising a deletion, substitution and/or addition of one or several nucleotides with respect to the nucleotide sequence shown in SEQ ID NO: 10, and encoding a fluorescent protein.

24. A DNA of either one of the following:

- (a) DNA which encodes the amino acid sequence shown in SEQ ID NO: 11, 13, 15 or 17;
or
- (b) DNA having a nucleotide sequence shown in SEQ ID NO: 12, 14, 16 or 18.

25. A recombinant vector having the DNA of any of claims 21 to 24.

26. A transformant having any of the DNA of any of claims 21 to 24 or the recombinant vector of claim 25.

27. A fusion fluorescent protein consisting of the fluorescent protein of any of claims 15 to 20 and another protein.

28. The fusion fluorescent protein of claim 27 wherein said another protein is one that localizes in the cell,.

29. The fusion fluorescent protein of claim 27 or 28 wherein said another protein is one specific to an intracellular organella.

30. A method for analyzing the localization or dynamics of a protein in cells, characterized in that the fusion protein of any of claims 27 to 29 is allowed to be expressed in cells.

31. A fluorescent reagent kit which comprises the fluorescent protein of any of claims 15 to 20, the DNA of any of claims 21 to 24, the recombinant vector of claim 25, the transformant of claim 26, or the fusion fluorescent protein of any of claims 27 to 29.